[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY]

α- and β-Spinastenol

BY DONALD LARSEN AND FREDERICK W. HEYL

Three isomeric sterols have been isolated from the unsaponifiable fraction of spinach.[1,2,3] As in the case of the sterols in ergot, we have found that upon catalytic hydrogenation each isomeric sterol of spinach yields one and the same reduction product; the differences in the original isomers reside only in the unsaturated linkages and are removed by the addition of two atoms of hydrogen.

Ozonization of α-spinasterol acetate was carried out by the method of Guiteras, Nakamiya and Inhoffen[4] and it was established definitely that the side chain of this sterol is saturated; *i. e.*, both unsaturated linkages reside in the condensed rings.

Since the first reduction product still retains a difficultly reducible unsaturated linkage, it has been named α-spinastenol. Furthermore, since it can be isomerized to a form which is readily and completely reducible, we distinguish here between α- and β-spinastenol, the latter corresponding to β-ergostenol in the ease with which it adds the last two atoms of hydrogen.

α-Spinastenol acetate melts at 119°, $[\alpha]_{5461}$ +11.6; the respective sterol melts at 110°, $[\alpha]_{5461}$ +24.2. β-spinastenol acetate melts at 86°, $[\alpha]_{5461}$ +24.3; the respective sterol melts at 127°, $[\alpha]_{5461}$ +36.5.

The striking resemblance between the α- and β-ergostenols and the α- and β-spinastenols is extremely interesting, especially as they each belong to the C_{28} series. Table I shows the properties of known singly unsaturated sterols which are isomerized to the β-form and are then further reducible to the saturated sterol alcohol.

TABLE I

		Sterol			Acetate		
Compound	Ref.	M. p., °C.	Rotation		M. p., °C.	Rotation	
α-Ergostenol	[a]	131	$[\alpha]_D$	+11.4°	110	$[\alpha]_D$	0
β-Ergostenol	[b]	141	$[\alpha]_D$	+21.2°	114	$[\alpha]_D$	+10°
γ-Ergostenol	[c]	146	$[\alpha]_D$	0	157	$[\alpha]_D$	− 5.3°
α-Dihydro-zymosterol	[d]	122	$[\alpha]_{5461}$	+25.4°	84	$[\alpha]_{5461}$	+11.4°
β-Dihydro-zymosterol	[d]	100	$[\alpha]_{5461}$	+32.1°	75	$[\alpha]_{5461}$	+15.1°
α-Spinastenol	[e]	110	$[\alpha]_{5461}$	+24.2°	119	$[\alpha]_{5461}$	+11.6°
β-Spinastenol	[e]	127	$[\alpha]_{5461}$	+36.5°	86	$[\alpha]_{5461}$	+24.3°

[a] A. Windaus and A. Lüttringhaus, *Ann.*, **481**, 119 (1930). [b] M. C. Hart and H. Emerson, THIS JOURNAL, **54**, 1070 (1932). [c] A. Windaus and R. Langer, *Ann.*, **508**, 105 (1933). [d] F. Reindel and A. Weickmann, *ibid.*, **475**, 86 (1929). [e] This paper.

The properties of the saturated alcohols derived from the above β-forms are listed in Table II; sitostanol, although C_{29}, is also included. Remarkably close agreement in the properties of these saturated alcohols is evident.

TABLE II

		Sterol			Acetate		
Compound	Ref.	M. p., °C.	Rotation		M. p., °C.	Rotation	
Allo-α-ergostanol	[a]	144	$[\alpha]_D$	+15.4°	146	$[\alpha]_D$	+ 8.0°
Ostreastanol (Sitostanol)	[b]	141	$[\alpha]_D$	+23.7°	137	$[\alpha]_D$	+14.3°
Zymostanol	[c]	138	$[\alpha]_{5461}$	+20.6°	131	.....	
Spinastanol	[d]	137	$[\alpha]_{5461}$	+27.8°	132	$[\alpha]_{5461}$	+16.3°

[a] M. C. Hart and H. Emerson, THIS JOURNAL, **54**, 1070 (1932). [b] W. Bergman, *J. Biol. Chem.*, **104**, 553 (1934). [c] F. Reindel and A. Weickmann, *Ann.*, **475**, 86 (1929). [d] This paper.

Work is in progress comparing the structure of allo-α-ergostanol with spinastanol.[5]

Experimental

α-Spinasterol.—α-Spinasterol was isolated from the spinach unsaponifiable as described by Hart and Heyl.[1] It was purified by repeatedly recrystallizing from alcohol at 60°. It was then recrystallized six times from ether. The acetate, *p*-nitrobenzoate and phenylurethan were prepared in the usual manner.

α-Spinastenol.—Fifteen grams of α-spinasterol acetate was hydrogenated with Adams platinum catalyst in acetic acid. The recovered mixture after two crystallizations gave about 10 g. of α-spinastenol acetate of m. p. 118–119°; recovery of the filtrates and further hydrogenation with fresh catalyst gave about 3 g. more of α-spinastenol acetate. The melting point remained constant even after again shaking with hydrogen and catalyst at 60–70°. We were unable to make the benzoate, esterification failing to take place; the sterol was recovered unchanged.

β-Spinastenol.—Ten grams of α-spinastenol acetate was isomerized by the directions of Heilbron,[6] except that we employed the acetate instead of the benzoate; as stated above the α-spinastenol would not esterify with benzoyl chloride; 10 g. of α-spinastenol acetate was dissolved in 20 cc. of dry chloroform, maintained at 0° in an ice-bath, and dry hydrogen chloride bubbled through the solution for two hours. The chloroform solution was washed with dilute bicarbonate solution and evaporated *in vacuo* with

(1) M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, **95**, 311 (1932).
(2) F. W. Heyl and D. Larsen, *J. Am. Pharm. Assoc.*, **22**, 510 (1933).
(3) F. W. Heyl and D. Larsen, THIS JOURNAL, **56**, 942 (1934).
(4) Guiteras, Nakamiya and Inhoffen, *Ann.*, **494**, 116 (1932).
(5) C. K. Chuang, *Ann.*, **500**, 277 (1933).
(6) I. M. Heilbron and D. G. Wilkinson, *J. Chem. Soc.*, 1708 (1932).

the least necessary warming. The residue could not be separated into the α- and β-isomeric acetates by fractional crystallization. Ten or twelve of the more common solvents, and various mixtures of them, were tried without success. Successive crops all melted at 80–82°, $[\alpha]_{5461}$ +21–23°. The various fractions were joined and saponified in 5% alcoholic potassium hydroxide. The isomeric sterols failed to be separated by recrystallizing from any solvent or mixture of solvents. Successive crops melted consistently at 95–105°, $[\alpha]_{5461}$ +33–34°. By observing the rotations of α-spinastenol, its acetate, β-spinastenol and its acetate, it can be seen that isomerization had taken place in about 80% yield.

The eutectic mixtures of the isomeric sterols were joined and converted to the *p*-nitrobenzoates. This mixture was recovered and taken up in dry ether. Successive crops melted at 180–181°, 179–180°, 190–192°. The two top fractions were joined and recrystallized from ether, m. p. 182.5–183°; no change by recrystallizing from other solvents; yield about 40%. Micro-hydrogenations absorbed 1.10, 0.90 and1.04 moles of hydrogen. Saponification gave β-spinastenol, melting sharply at 127°. Acetylation gave the acetate, melting at 86°.

Spinastanol.—Three grams of β-spinastenol acetate was hydrogenated in acetic acid with Adams catalyst. The acetic acid was removed under reduced pressure and the residue recrystallized from acetone; melting point constant at 131–132°; yield 80%. Hydrogenation of the filtrates with fresh catalyst raised the yield to nearly 100%.

Bromination of α-Spinasterol Acetate.—Two grams of α-spinasterol acetate was dissolved in chloroform, chilled to 0° and a slight excess of 2 *N* bromine in chloroform slowly added. Substitution occurred very abundantly. No dibromide could be isolated from the exceedingly soluble mixture. The reaction was tried also in carbon tetrachloride solution and with carbon tetrachloride and acetic acid solutions of bromine. No bromide could be isolated. Evaporated solutions invariably resulted in oils.

Ozonization of α-Spinasterol Acetate.—A preliminary ozonization was carried out on ergosterol acetate by the method of Guiteras.[4] The recrystallized semicarbazone melted at 130–131°, yield about 35%.

Ten grams of α-spinasterol acetate was suspended in acetic acid and treated exactly as above. It failed to go into solution, indicating that ozonization was not taking place. Ozone was passed through for two hours, water and zinc dust added, steam distilled, but no oil came over. The distillation residue was extracted with ether, the ether evaporated and the residue recrystallized from ethyl acetate–alcohol. α-Spinasterol acetate was recovered unchanged; loss of about 20% of material, probably due to ozone attacking one of the nuclear double bonds.[7]

Perbenzoic Acid Titrations.—The sample to be titrated was dissolved in 2 cc. of chloroform, enough chloroform solution of the perbenzoic acid added to make a 100% excess, and allowed to stand in the ice box for two to five days. Blank solutions of the acid were also determined over the same period. Unused acid was determined with acidified potassium iodide solution and sodium thiosulfate.

Saponification Values.—The respective esters were boiled for one hour in 25 cc. of 0.1 or 0.2 *N* 95% alcoholic potassium hydroxide. Excess alkali was determined with 0.1 *N* hydrochloric acid. Blanks were run in parallel and the acetylation values computed in the usual manner.

Specific Rotations.—Samples of 0.3–0.5 g. were used, dissolving in 10 cc. of chloroform. Rotations were taken in a 2-dm. all-glass tube, λ = 5461. The temperature was always 20 ± 1°.

TABLE III

Compound	Double bonds	M. p., °C.	$[\alpha]_{5461}^{19-21}$	Formula	Analyses: Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Molecular weight Calcd.	Molecular weight Found
α-Spinasterol	2	172.5	− 3.7	$C_{28}H_{46}O$	84.3	84.3	11.6	11.6			398	
Acetate	2	187	− 5.8	$C_{30}H_{48}O_2$	81.7	81.6	11.0	11.2			440	437 439 442
p-Nitrobenzoate	2	220	+ 5.2	$C_{35}H_{49}O_4N$	76.7	76.8	9.0	9.1	2.5	2.5	547	545 549
Phenylurethan	2	177	− 3.3	$C_{35}H_{51}O_2N$	81.2	81.2	9.9	10.1	2.7	2.7	517	518 519
α-Spinastenol	1	110	+24.2	$C_{28}H_{48}O$	83.9	83.7	12.1	12.2			400	
Acetate	1	119	+11.6	$C_{30}H_{50}O_2$	81.4	81.5	11.3	11.5			442	437 441 445
p-Nitrobenzoate	1	192	+ 6.6	$C_{35}H_{51}O_4N$	76.5	76.6	9.4	9.6	2.5	2.4	549	540 545 548
β-Spinastenol	1	127.5	+36.5	$C_{28}H_{48}O$	83.9	83.9	12.1	12.1			400	
Acetate	1	86.5	+24.3	$C_{30}H_{50}O_2$	81.4	81.2	11.3	11.3			442	439 442
p-Nitrobenzoate	1	183	+37.3	$C_{35}H_{51}O_4N$	76.5	76.7	9.4	9.5	2.5	2.7	549	549 551
Spinastanol	0	137	+27.8	$C_{28}H_{50}O \cdot 0.5H_2O$	81.7	81.7	12.6	12.6			411	
Acetate	0	132	+16.3	$C_{30}H_{52}O_2$	81.0	81.1	11.8	11.8			444	438 441 448
p-Nitrobenzoate	0	213.5	+24.4	$C_{35}H_{53}O_4N$	76.2	76.2	9.7	9.9	2.5	2.7	551	548 550 553
Phenylurethan	0	172	+16.4	$C_{35}H_{55}O_2N$	80.6	80.6	10.6	10.8	2.7	2.7	521	518 522

The derivatives in every case were saponified and the recovered products showed substantially the same melting points and rotations as the original substances.

Summary

1. The double bonds of α-spinasterol are shown to be in the nucleus.

2. Hydrogenation yields α-spinastenol, which is not further reducible directly but is readily isomerized to β-spinastenol in a manner strictly analogous to the isomerization of α-ergostenol to β-ergostenol.

(7) A. Windaus and H. H. Inhoffen, *Ann.*, **510**, 260 (1934).

3. β-Spinastenol yields a saturated alcohol, spinastanol, on absorption of one mole of hydrogen.

4. New esters of α-spinastenol, β-spinastenol and spinastanol are described.

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Preparation of 1,3-Diketones by the Claisen Reaction

BY JAMES M. SPRAGUE, LELAND J. BECKHAM AND HOMER ADKINS

Rather large quantities of a number of 1,3-diketones were needed in this Laboratory for studies involving the relation of their structures to the mode of cleavage by water, alcohol and hydrogen.[1] Our experience in the preparation of these diketones (see Table I) by the condensation of an ester with a simple ketone according to the Claisen reaction has, we believe, illuminated somewhat the preparational process.

Sodium ethoxide, especially by the procedure marked "A" in the experimental part, appears to be preferable to sodium as a condensing agent.[2] Only in the case of diacetylmethane was sodium definitely superior with respect to the yield of products calculated upon the basis of the amount of monoketone used. If the yields were calculated upon the basis of the sodium ethoxide or sodium the comparison would be in all cases very unfavorable to sodium since as shown by Kutz 2 g. atoms of sodium are required as contrasted with 1 g. molecule of sodium ethoxide.

Even though sodium gave a higher percentage conversion of monoketone to diketone, the reagent suffers under certain disadvantages that greatly militate against its use. The condensation of an ester with a ketone using sodium as the condensing agent is a hazardous operation. One serious accident and several minor ones have occurred in this Laboratory in connection with the preparation. The serious accident was due to contributory negligence upon the part of the operator but even the most skilled and experienced occasionally allow a reaction to become too violent. The difficulty lies in the fact that usually reaction occurs slowly if at all when the reactants are mixed at 0–5°, but it does begin in most cases as the mixture warms up to room temperature. Once under way the reaction goes rapidly and unless the reaction mixture is watched very carefully, and rapidly cooled, goes with violence. It is, of course, not difficult to use sodium if only a fraction of a mole is involved.

The formation of a β-keto ester through the interaction of two molecules of ester may be avoided to a greater extent with sodium ethoxide than with sodium. In certain attempted preparations of $CH_3C(O)CH_2C(O)C(CH_3)_3$ through the condensation of ethyl acetate and pinacolone with sodium, a product having a good boiling range for the diketone contained as much as 50% acetoacetic ester while that from sodium ethoxide contained no ester. Sodium also tends to produce acyloins. For example no diketone was obtained as a result of the use of sodium with ethyl hexahydrobenzoate and acetone, instead the acyloin, $C_6H_{11}C(O)CH(OH)C_6H_{11}$, was isolated in 70% yield accompanied by a 5–6% yield of dicyclohexylglycolic acid.

Three methods are available for the isolation and purification of the diketone. The purification is readily made if the diketone is a solid such as are acetylbenzoylmethane, furoylbenzoylmethane, etc., which precipitate upon acidification of the reaction mixture. Morgan and others have recommended the separation of the liquid diketones as the copper salts. However, we have found that in most cases it suffices to extract the diketone from an acid solution and then to purify it by fractionation through a Widmer column. Presumably the preparation of the copper salt of the diketone is intended to eliminate any β-keto ester present in the reaction product. It is unnecessary to form the copper salt, (a) if no β-keto ester is present, or (b), if the β-keto ester differs sufficiently in boiling point from the diketone so that it may be separated by fractionation. Analysis of the products has shown, for example, that isovaleryl-, trimethylacetyl- and

(1) For references see Sprague, Beckham and Adkins, THIS JOURNAL, **56**, 2669, 2676 (1934).

(2) Nothing in this paper should be construed as in conflict with the conclusion of McElvain and others that the acetoacetic ester or Claisen condensation is dependent upon sodium ethoxide even when sodium is the reagent added.